THE BACTERIAL ORIGIN OF THE GUMS OF THE ARABIN GROUP.

XI.—The Nutrition of *Bacterium acaciæ*.

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(Plates xi.-xii.)

In the first two papers of this series it was shown that when *Bact. acaciæ* and *Bact. metarabinum* were sown upon the surfaces of plates of saccharose-potato-tannin agar, they produced luxuriant slimes, and that from the slimes arabin and metarabin could be obtained. Since the medium produced so much slime it was extremely probable that it contained the nutrients which were best adapted to assist the bacteria in producing gum. The medium consisted of saccharose 5%, tannin 0·3%, agar 2%, and potato-juice or extract. The latter is the unknown quantity. It would be necessary to determine the composition of the potato-juice to arrive at a knowledge of the nutrition of the bacteria, and two methods might be adopted for this purpose. Either the juice may be analysed, or one might experiment with various single nutrients in the hope of finding a clue which would lead to the desired end. I chose the latter alternative, chiefly because by doing so there was the probability of increasing the yield of slime by using nutrients which might not be in potato-juice.

Preliminary experiments.—According to Dammar and Rung (Chemisches Handwörterbuch), potato-extract should contain reducing sugars, asparagine, traces of amido-acids, peptone, solanine and gums. The albuminoids would be removed during the preparation of the extract. The chief of these nutrients are the reducing sugars and the asparagine; the other constituents

might be neglected. But since saccharose was found to be useful in the saccharose-potato-tannin medium, the reducing sugars of the extract might also be neglected. There remains then asparagine as being the essential nutrient of the potato-extract. The salts of the extract would also doubtless assist the bacteria.

With this information at my disposal I prepared a saccharose-asparagine-tannin medium and infected plates of it with the bacteria. At the same time a comparative test was made with a medium identical with the other, excepting that it contained no tannin.

	Saccharose-asparagine-agar	
	without tannin.	with tannin.*
Bact. acaciæ (plum) ..	dry growth; no slime	thin non-adherent slime
Bact. acaciæ (almond) ...	scanty growth; no slime	thin non-adherent slime
Bact. metarabinum ...	dry growth; no slime	dry growth; no slime

It is evident that tannin was of considerable importance, not only in causing a production of slime, but also in making the slime non-adherent, an important point in the artificial production of the carbohydrate. The quantity of slime, however, was small compared with what was obtained when potato-extract had been employed. The absence of slime upon the plates infected with *Bact. metarabinum* is peculiar, and shows that the nutrition of this organism may differ from that of *Bact. acaciæ*, and that these bacteria would need to be investigated separately.

Believing that the salts of the last medium had had an influence in lessening the yield of slime, an experiment was made in which the medium in the tubes was treated with varying quantities of potassium chloride, potassium monohydrogen phosphate, and potassium citrate. The last-named salt was suggested by some earlier experiments, which indicated increased slime-yields by

* Saccharose 5, asparagine 0·1, tannin 0·3, KH_2PO_4 0·1, KCl. 0·5, agar 2, water 100 grms. Medium made neutral to litmus before the addition of the tannin.

potassium phosphate, citrate or asparaginate when peptone was used. Furthermore, citric acid is the chief organic acid in potato-juice, a fact which was brought out during the investigation of the acids formed by the bacteria.

Like the previous experiment the slime-production was scanty, but the results showed broadly that no slime was formed in the presence of chloride or phosphate. Slime was produced on the media containing citrate or citrate and chloride. A mixture of citrate and phosphate prevented slime-formation. It was made evident that a good salt to employ in the synthetic medium would be potassium citrate.

After finding that tannin and citrate were useful for a slime-producing medium, the next step which was taken was the influence of different carbohydrates. From 0·5-1 grm. of carbohydrate was dissolved in 20 c.c. portions of a medium containing peptone, citrate and agar; and with these plates were prepared, and the surfaces smeared with *Bact. acaciæ*. At the same time another set was made; these differed in containing asparagine in place of peptone. The results showed that levulose was the chief sugar from which the organism formed its slime, and that asparagine was much better than peptone.

Experimental methods.—Hitherto the quantity of slime produced by a particular combination of nutrients had been roughly estimated. But after making many experiments, it became apparent that some definite method would have to be adopted to distinguish small differences, and the use of the balance suggested itself. Before proceeding to consider the next experiment, perhaps it would be advisable to describe the method employed in obtaining a certain weight of slime. In preparing the medium the agar was first dissolved in about half the required quantity of water. The remainder of the nutrients, etc., were dissolved in rather less than half, which was heated and added to the solution of agar upon its removal from the autoclave. The incomplete medium was then put into wide (6 × 1 inch) numbered test tubes by means of a 200 c.c. burette graduated in cubic centimetres. Each tube contained 20 c.c. of medium. The nutrient under

experiment was weighed and put into the tube, which was shaken until solution had taken place. The tubes were next sterilised by being steamed once for three-quarters of an hour. The tannin medium is not a suitable pabulum for the majority of bacteria, so that one steaming is practically sufficient. Only once or twice have I seen a mould upon the plates, and many hundreds of these have been prepared. After steaming, the tubes were cooled to 50° and poured into 9 cm. Petri dishes. The infecting culture was prepared by growing *Bact. acaciæ* upon the sloped surface of saccharose-potato-agar at 30° for 24-48 hours. This produced a loose aggregation of cells without slime. Some of the culture was picked up with the front of a loop of stout platinum wire and smeared completely over the surface of the agar in the Petri dish. After 24 hours the lid of the dish was raised and the slime uniformly distributed with the same large platinum loop, which had an internal diameter of 4 mm. At the end of three days the slime was scraped by means of a small rubber spade into a counterpoised watch glass and weighed. Two days afterwards the slime which had formed during the interval was similarly removed and weighed. The plates were kept under observation for another two days to be sure that slime-production had ceased. The slimes were weighed upon an open coarse balance to the second decimal place, although the balance was sensitive to 5 milligrams. In the tables that follow, the total weights obtained are multiplied by 5 and expressed as the nearest whole number, thus giving the weight of slime in grams from 100 c.c. of medium. In one or two tables the actual weighings are recorded to show how the bulk of the slime is formed in the first three days.

Several experiments were made during the course of the research, when duplicate plates were used, to see if a larger quantity of infecting material had any tendency to increase the amount of slime. The results showed that the quantity of material had no influence upon the weight of slime. In some cases a little more was obtained, in others a trifle less, in others the weights were the same.

The influence of levulose.—Having found that levulose was a suitable sugar, an experiment was made to determine the most favourable quantity. The figures given are the percentages obtained at the end of the third day. Asparagine was the nitrogenous nutrient in the medium.

THE SLIME FROM VARYING QUANTITIES OF LEVULOSE.

Percentage of levulose.	Grams of slime from 100 c.c. of medium.*
None.	0
1	14
2	17
4	15
6	13
8	10
10	7

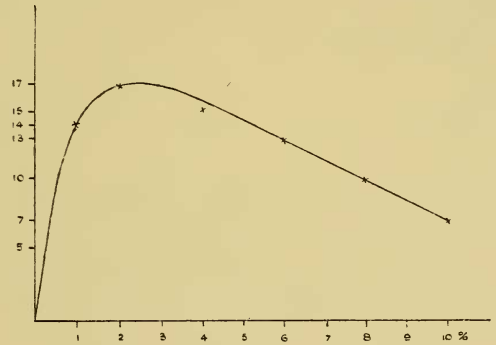


Fig. 1.—THE INFLUENCE OF LEVULOSE.

It is evident that the optimum percentage of levulose lies between 2 and 4 per cent. Upon plotting the numbers it is found that the yield reaches a maximum with about $2\frac{1}{2}$ % of levulose. The depressing effect of larger quantities is clearly shown.

The influence of salts.—The saline constituents of the medium are undoubtedly of considerable importance, and it has been

* Asparagine 0·3, potassium citrate 0·6, tannin 0·3, agar 2, water 100 grms.

already mentioned that citrates favoured, while chlorides and phosphates prevented, slime-formation upon a certain medium. A small quantity, 0·12 grm., of various salts was added to 20 c.c. portions of medium in tubes, so that the salts were present to the extent of 0·6 %. As a rule salts of potash were added, but in some cases the sodium or ammonium salt chanced to be the only compound in the laboratory.

The Influence of various Salts.

Salt.	Grams of slime from 100 c.c. of medium.*
Sodium succinate...	20
Potassium citrate...	18
Sodium potassium tartrate	15
No salt	15
Potassium chloride	14
,, sulphate	12
,, monohyd. phosphate	11
,, oxalate	9
Sodium acetate	0
,, formate	0
Ammonium lactate	0

It is seen from the table that the acid radicles may (1) accelerate, (2) be indifferent, (3) depress or (4) prohibit. Succinate and citrate favour the production of slime, and in the majority of the experiments in this research one or other of these salts has been employed. Tartrates are indifferent, while sulphates, phosphates and oxalates are depressants.

Among the acids which the bacterium forms from saccharose are acetic, lactic and succinic. It is interesting to note that of the three the first two prohibit and the last stimulates the gum-forming faculty of the organism.

Having found that citrate and succinate were stimulants, the next step was to discover the optimum quantities of these salts. Accordingly, tubes of media were prepared and various quantities of salt added.

* Asparagine 0·1, levulose 2, tannin 0·3, agar 2, water 100 grms.

INFLUENCE OF THE QUANTITY OF SALT UPON THE YIELD OF SLIME.

Salt.	Slime in grams from 100 c.c. of medium.*
No salt	25
Sodium succinate 0·1%	25
,, ,, 0·3	22
,, ,, 0·5	21
,, ,, 0·75	21
Potassium citrate 0·1	26
,, ,, 0·3	24
,, ,, 0·5	21
,, ,, 0·75	22

From the numbers as a whole we must conclude that succinates and citrates in anything but small amount, viz. 0·1%, act as depressants. By the reversal of the yields, as compared with the last experiment, the question is still undecided as to whether citrate or succinate is the better; although when the results are expressed in the graphic form the curves show that citrate is rather better than succinate.

The action of tannin.—Previous experience had shown that tannin increased the amount of slime, and at the same time made it of such a nature that it could be removed from the surface of the agar medium with the greatest ease. The very early experiments had indicated that the optimum quantity of tannin lay between 0·2 and 0·4%, and 0·3% was taken in making the saccharose-potato media. That this is the best quantity is open to doubt, and an experiment was arranged to find the weights of slime produced by varying amounts of tannin in a synthetic medium. Tannin is objectionable for two reasons. Firstly, it tends to soften the medium during sterilisation. This it probably does by reason of its acidity. Secondly, it passes into the slime and darkens the gum which is ultimately obtained unless the solutions are made decidedly acid, a condition which somewhat hinders the precipitation of the gum by alcohol. If, therefore, smaller quantities of tannin should be found to be as good as larger for the purpose, a distinct advance would be made.

* Asparagine 0·1, levulose 2, tannin 0·1, agar 2, water 100 grms.

The slimes in the experiment were incubated at 13° and weighed at the end of the third day.

THE SLIME FROM VARYING AMOUNTS OF TANNIN.

Tannin %.	Grams of Slime from 100 cc. of medium.*
None.	10
0·1	16
0·2	16
0·3	16
0·3 (new lot).	11

The influence of the tannin in giving an easily removable slime was again demonstrated, although the difference was not so marked as in the earlier experiments, especially with saccharose-potato-agar. The better nutrition of the bacterium, *e.g.*, with levulose, caused a greater slime-production, with a concomitant greater ease in removal. The consistency of the plate containing 0·1% was firmer and the medium of a lighter colour. Both of these appealed strongly in favour of the use of 0·1%. However, I determined to repeat the experiment, especially as the test with a new lot of tannin gave such a very low yield. In this experiment which follows, the temperature of the laboratory varied from 14° to 17° C. The old and new lots of tannin were powders; the commercial sample consisted of dark-coloured lumps.

THE EFFECT OF DIFFERENT TANNINS.

Tannin.	Slime in 3½ days, grams.	Slime 2 days later, grams.	Total Slime from 100 c.c. of medium† grams.
None	4·48	0·23	24
Old lot 0·1%	4·55	0·15	24
,, 0·3%	4·40	0·16	23
New lot 0·1%	4·14	0·16	22
,, 0·3%	4·08	0·11	21
Commercial lump 0·3% ...	4·61	0·60	26
Gallic acid 0·3%	1·17	0·13	7
Pyrogallol 0·3%	(0·05)	0·00	0

* Asparagine 0·1, levulose 2, potassium citrate 0·6, agar 2, water 100 grms.
† Asparagine 0·1, levulose 2, sodium succinate 0·5, agar 2, water 100 grms.

The most notable result of the experiment is the finding that different tannins produce different results. The smaller quantity, viz., 0·1%, produced more slime than the larger percentage. Plates containing 0·5% were also prepared, but the medium was like starch paste; its gelatinous property had been destroyed by the acid. All the plates containing tannic and gallic acid yielded slimes which were readily removed. The slime in the plate without tannin was not homogeneous and apparently contained zoogloea masses, but still it could be removed without taking away particles of the medium.

The function of the tannin.—One cannot say what the rôle of the tannin in the synthetic medium may be. It is possible that it acts by reason of its acidity, or it may form an unstable compound with the levulose, and thus play the part of a catalyte. Probably it does neither of these, but before discussing its function it would be advisable to record some experiments with organic acids, and with salts and tannin. The acidity of 0·1 % of tannin is approximately equal to 0·02 % of citric or succinic acid, so this quantity was used in order to compare the effect of the free acids.

THE EFFECT OF ACIDITY AND OF TANNIN.

	Grams of Slime in five days from 100 c.c of medium.*	
	Experiment 1.	Experiment 2.
Neither salt nor tannin	16, 16	19
Succinic acid, 0·02%...	18	16
Citric acid, 0·023%	17	17
Tannic acid, 0·1%	17	19
Potassium citrate, 0·1% + tannin 0·1%	17	20
Potassium succinate, 0·1% + tannin 0·1% ...	17	...
,, ,, ,, ,, 0·05 ...	17	...
,, ,, ,, ,, 0·02 ...	16	...

* Asparagine 0·1, levulose 2, agar 2, water 100 grms.

Experiment 3.

	Slime on					Grms. of Slime from 100 c.c. of medium.*
	3rd day.	5th day.	Reinfected.	10th day.	12th day.	
Succinic acid ...0·02%	3·80	0·06		0·22	0·00	20
Citric acid . 0·02	3·07	0·05		0·47	0·00	18
Commercial tannin 0·1	3·79	0·29		0·00	0·00	20
Comm. tannin, 0·1% + Pot. citrate 0·1% ...	4·30	0·59		0·03	0·00	25
Potassium citrate, 0·1%	3·35	0·02		1·32	0·47	26
Neither acid nor salt ...	3·80	0·02		0·66	0·00	22

The three experiments do not agree in details. The divergences are partly due to the different temperatures of incubation, possibly also to a difference in the vitality of the bacterium at the time of each experiment, for they were not made simultaneously, but following each other. But the third experiment shows that a reinfection of the media was necessary to enable the nutrients in the absence of tannin to be utilised. It is clear that the tannin does not act by reason of its acidity. It seems to act as a stimulant, for the nutrients are quickly utilised, that is to say, the slime is formed quickly. In its absence the gum is very slowly produced, as can be seen by referring to the action of potassium citrate and comparing it with that salt plus tannin. Citric acid alone made the medium brittle, so that the infection of the plate and the removal of the slime had to be done with the greatest care to prevent angular pieces of the agar coming away with the slime. In considering the experiments as a whole, one sees that a pronounced acidity of the medium is not to be recommended. The addition of tannin and citrate is advisable, but in the presence of a suitable proportion of asparagine and levulose the addition could almost be dispensed with. If after adding citrate only the medium is reinfected with bacteria, the maximum yield of slime can be obtained.

With regard to the function of the tannin in the medium, I am of the opinion that it is purely physical and causes the agar jelly to be somewhat contractile. A splitting of the agar is a

* Asparagine 0·1, levulose 2, agar 2, water 100 grms.

common phenomenon when large plates are infected. A contractile jelly would slowly supply the bacteria with fluid containing the nutrients. As the slime contains about 97% of water, from 20 to 24 grams of water are taken from every 100 grams of agar medium. The agar without tannin has probably as great an affinity for this water as has the slime, in which case the slime would not be able to get the necessary amount. But the tannin, by slightly contracting the agar or lessening its affinity for water, enables the bacteria to get the moisture slowly and in quantity sufficient for a maximum formation of slime.

The action of various tannins.—The experiments with different tannins showed that they varied in their effects; some increased while others diminished the yield of slime. With the object of determining the action of known tannins, I obtained samples from Messrs. Harrington Bros., of London and Cork, and subjected these to experiment. The results are expressed in the following table.

The Influence of different Tannins.

Tannin.	Slime in grams from 100 c.c. of medium in three days.			in six days.		
	Experiment i.*	ii.*	Glycerine†	Experiment. i.	ii.	Glycerine.
1. G. powder	19, 18	15, 15	18, 18	21, 19	17, 17	22, 21
2. G. granular	19, 18	17, 16	18, 17	21, 20	20, 18	23, 21
3. Commercial 6713 ..	19, 17	15, 15	17, 16	20, 18	17, 17	23, 22
4. Commercial	16, 16	15, 15	16, 16	18, 18	17, 16	21, 21
5. No tannin	11, —	10, 10	18, 17	13, —	13, 13	19, 18
6. Granular 6087	9, 8	10, 9	14, —	11, 9	15, 13	20, —
7. Extra cryst.	9, 8	10, 8	16, 14	11, 9	13, 12	22, 21
8. W. B. powder	9, 9	7, 7	12, 11	11, 11	13, 11	19, 18
9. Levissimus	6, 6	10, 9	15, 15	7, 7	12, 10	19, 17
10. Pure	9, 8	8, 7	13, 13	13, 10	12, 11	21, 21

For some reason the duplicate tests did not agree so closely as could have been wished, and as they differed in some cases by 2 or 3 units, I have given the numbers as they were

* Levulose 2, (tannin 0·1), potassium citrate 0·1, asparagine 0·1, agar 2, water 100.

† Levulose 2, glycerine 1, (tannin 0·2), potassium citrate 0·1, asparagine 0·1, agar 2, water 100.

obtained, although it would have perhaps been more striking had only certain numbers been given, as, for example, the higher of two duplicates upon the third day. Experiment No. 1 cannot be compared with No. 2 because the experiments were made at a time when the laboratory temperature differed considerably from day to day; each experiment must be considered by itself.

Considering the experiments broadly, it is clear that certain tannins augment and that others diminish the yield of slime. Purity appears to be a hindrance, from which it would appear that the stimulative or the physical effect, whichever it be, is due to the presence of an impurity. Tannin No. 3 arrived at the laboratory in the same condition as the commercial lot with which the other experiments were made; the passage through the tropics had caused the powder or granules to fuse into hard glistening dark lumps. They were probably the same. Nos. 1, 4 and 8 were fine buff-coloured powders, very similar in appearance; No. 10 was much the same, but coarser. Nos. 2 and 6 were granular and glistening, 2 being dark, 6 being light in colour. No. 4 consisted of dull, light-coloured granules. Messrs. Harrington Bros. informed me that Nos. 1, 2 and 3 were prepared specially for dyers and were manufactured from sumach; Nos. 6, 7 and 8 for printers, and No. 10 (pure) were obtained from gall nuts.

At present I cannot explain the difference between 1, 2, 3 and 6, 7, 8, but it would seem that *Bact. acaciæ* can enable the tannins to be separated into two groups. One group considerably augments the yield of slime and includes the sumach tannins used by dyers. The other group either is inactive or diminishes the yield. It contains the kinds obtained from gall nuts, such as the purer forms and those used by calico printers. Since *Bact. acaciæ* can distinguish between certain of the tannins it is possible that it might form the basis of a biological method for their recognition.

The experiment with glycerine shows that this substance assists the tannin, so that by their combined use any depressing effect of one is annulled if sufficient time be allowed for the complete growth of the slime.

The solids of the slime.—Believing that the amount of solid matter in the slimes might vary considerably and thus account for some of the discrepancies in the experiments, I determined the percentage of water in the slimes obtained from a temperature experiment.

THE WATER CONTENT OF THE SLIME.

Incubation temperature.	Total Slime from 20 c.c. of medium, grams.	Water in Slime %.
14-15°	4·2	96·8
22°	3·6	97·2
22° (duplicate)	3·6	97·2
30°	1·0	96·4

These figures show that the percentage of water in the slime is very constant, and that a smaller yield of slime does not necessarily involve a higher percentage of dry matter.

The influence of asparagine.—Early in the research the effect of varying percentages of different nitrogenous nutrients was tested in a saccharose medium, but as this was contained in tubes only general conclusions could be drawn from the growths upon the sloped agar surfaces. Asparagine and urea produced a fair amount, peptone gave only traces, while the bacterium did not form slime in the presence of ammonium and potassium nitrates.

At a later period the influence of asparagine was quantitatively determined with the following results :—

THE INFLUENCE OF ASPARAGINE.

Asparagine %.	Grams of Slime from 100 c.c. of medium.*
None	0·5
0·005	2
0·015	4·5
0·025	8
0·05	16
0·10	24
0·15	23
0·20	22
0·25	23
0·3	24
0·4	23
0·5	24

* Levulose 2, comm. tannin 0·1, potassium citrate 0·1, agar 2, water 100 grms.

A similar experiment, in which some of the stages were destroyed, corroborated these results. It is seen that the bacterium

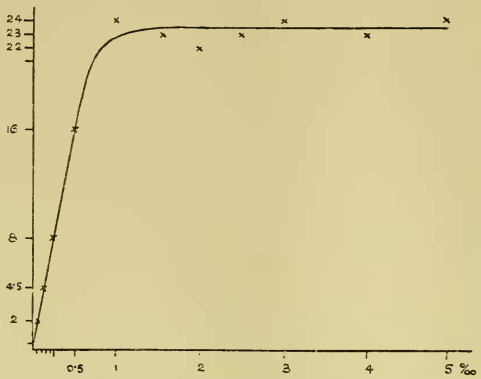


Fig. 2—The Influence of Asparagine.

responds quickly to the asparagine, and that the quantity suggested by the qualitative test, viz., 0·1%, is an optimum percentage.

The prohibitive action of dextrose upon levulose.—In a preliminary experiment made with the object of testing the action of asparagine against peptone with various sugars, it was noted that the plates containing invert sugar produced no slime. The experiment is duplicated elsewhere and need not be given, but the curious behaviour of the sugar warranted investigation. The following experiment was made :—

The Influence of Dextrose in Checking Slime-Formation.

	Grams of Slime in 3 days from 100 c.c. of medium.*
No sugar	0
Dextrose 1%	2
,, 2	2
Levulose 1	10
,, 2	14
Invert sugar 1%	2
,, ,, 2	1
Levulose 1% + dextrose 1%	3
,, ,, ...	3
,, ,, 2%	3

* Asparagine 0·1, potassium citrate 0·6, tannin 0·3, agar 2, water 100.

It is evident that the bacterium practically cannot form gum from dextrose, and also that this sugar is not inert in its action; it prevents the utilisation of levulose and consequent production of slime. The next step in connection with the action of dextrose was to see the effect of adding varying quantities of the aldose, dextrose, to the ketose, levulose.

THE DEPRESSING ACTION OF DEXTROSE UPON LEVULOSE.

	Grams of Slime from 100 c.c. of medium.*
No sugar	0
Levulose 2%	22
,, ,, +dextrose 0·25%	22
,, ,, ,, 0·5	21
,, ,, ,, 1·0	16
,, ,, ,, 1·5	11
,, ,, ,, 2·0	5

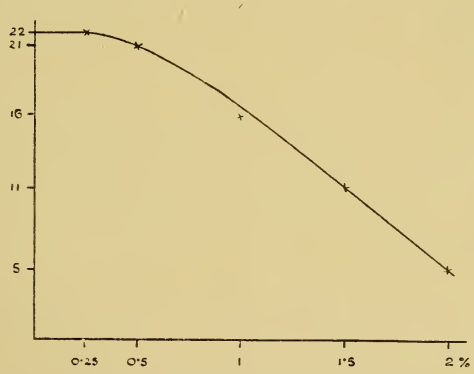


Fig. 3.—THE PROHIBITIVE ACTION OF LEVULOSE.

The depressing influence of dextrose upon levulose is again clearly shown. With a proportion of dextrose to levulose as 1 to 8, the dextrose has no depressing action, but as the ratio

* Asparagine 0·1, sodium succinate 0·5, tannin 0·1, agar 2, water 100.

becomes narrower the depression becomes more and more conspicuous.

The use of other sugars, etc.—The influence of other commonly occurring sugars and nutritive alcohols upon slime-formation is shown in the table which follows. The experiment was made at a time when the laboratory temperature was rather high (20°-22°), and this accounts for the poor yield with levulose as compared with the yields in other experiments.

The Influence of various Sugars, etc., upon Slime-formation.

	Slime in grams from 100 c.c. of medium.*
Saccharose	20
Levulose	19
Dextrose	0
Glycerine	21
Mannite	15
Maltose	11
Raffinose	0
Lactose	0
Galactose	0

The substances that aid slime-formation are seen to be levulose, saccharose, maltose, mannite and glycerine; and of these it is interesting to note the part played by glycerine. It is curious that maltose, which hydrolyses to dextrose, is a nutrient, while dextrose itself is not.

Various sources of carbon and nitrogen compared.—As it appeared possible that the nitrogenous food might influence the effect of these carbonaceous nutrients, an experiment was made in which asparagine, urea and peptone were compared. The incubation temperature was about 17°, the temperature of a refrigerator. The peptone, asparagine and urea were added in quantities containing approximately equal amounts of nitrogen.

* Asparagine 0·1, (sugars, etc., 2·0), tannin 0·1, potassium citrate 0·1, agar 2·0, water 100.

THE INFLUENCE OF DIFFERENT CARBONACEOUS AND NITROGENOUS CONSTITUENTS.

	Slime in grms. from 100 c.c. of medium* containing		
	Peptone.	Asparagine.	Urea.
Levulose	1	28	28
Saccharose	3	24	18
Maltose	4	24	15
Glycerine	4	20	13
Mannite	1	20	12
Dextrose	0	0	0

The most pronounced result is the small yields of the peptone media as compared with the amides, asparagine and urea. Asparagine is the better amide, and it is in all probability the form in which the bacterium obtains its nitrogen under natural conditions. At the same time it must be remembered that the bacteria under experiment had been subcultivated upon potato-extract agar, to the asparagine of which the bacteria must have become accustomed under artificial conditions.

The isolation of a fresh race of the bacterium.—The accustoming to certain nutrients by subcultivation upon certain media must be borne in mind when discussing the nutrition of the bacteria. We have seen that certain carbohydrates are more favourable to slime-formation than others, but the same results might not have been obtained had the organisms been taken directly from their natural habitat and sown upon the experimental medium. This is, of course, impossible, for the bacteria must first be purified, and this process involves the employment of artificial media. But much might be learned by minimising the time during which the bacteria are under the artificial conditions. With this idea portions of the branches of a specimen of *Acacia decurrens* were introduced into flasks containing a saccharose-peptone medium. After some time a gummy mass

* Tannin 0·1, potassium citrate 0·1, (sugars, etc. 2·0), (asparagine 0·1, peptone 0·1, urea 0·033), agar 2·0, water 100.

formed upon the branch in one of the flasks. This was extracted and smeared over the surface of a levulose-asparagine-tannin-agar plate. A slime developed and a portion (large loopful) was used for smearing another plate. The slime that grew was "plated" upon saccharose-potato-agar, and two of the colonies that developed were grown upon the same medium. These growths were smeared over plates of media containing various sugars, and at the same time the cultures were tested upon glucose-gelatine for purity and for their identification. The cultures were pure; one of them was *Bact. metarabinum*, and the other *Bact. acaciæ*. Upon the experimental media the former produced a dry leathery slime that could not be removed from the plates. The indications, however, were that levulose was the best carbohydrate and saccharose the next. *Bact. acaciæ* grew as a slime which was decidedly more viscous or ropy than were the races after much subculture. The weights of slime are given in the following table :—

SLIME-PRODUCTION BY A FRESHLY ISOLATED RACE OF *Bact. acaciæ*.

Incubation temperature 17°.	Slime in grms. from 100 c.c. of medium.*
Levulose	14
Saccharose	7
Dextrose	1 (thin watery fluid)
Maltose	3
Galactose	1 (thin watery fluid)
Mannite	8
Glycerine	9

The yields are not so great as had been obtained with the older bacterium. The new organism was probably a weaker race. It did not grow so strongly upon artificial media and was of a paler colour. The older race was of a deep yellow colour on saccharose-potato-agar; the new race was white, with a buff tinge.

The utilisation of saccharose and maltose.—The experiments with the organism and various sugars have shown that the gum

* Sugars 2, asparagine 0·1, tannin 0·1, potassium citrate 0·1, agar 2, water 100.

is formed from asparagine and levulose. The organism does not secrete invertase, and as it can be made to gain the power of utilising saccharose it is evident that this sugar must be used as such, and not as either or both of the products of hydrolysis. Furthermore, the yield of slime from saccharose when the bacterium is thoroughly accustomed to this sugar is very much greater than what is obtained from a mixture of dextrose and levulose. Maltose must also be utilised as such, for its product of hydrolysis, dextrose, is incapable of assisting gum-formation.

The gum is not derived from cellulose.—The production of the gum from levulose has a direct bearing upon certain hypotheses regarding the origin from cellulose.

Cellulose is a broad term and the statement is on that account sweeping. Under the term may be included substances of such diverse characters and composition as resistant cellulose, the hydrocelluloses, hemicelluloses, the pectins and the oxycelluloses.* The hydrolysed products of these bodies consist of one or more of the following sugars :—dextrose, levulose, mannose, galactose, arabinose, xylose, the furfuroïds of Cross, Bevan and Smith, and possibly others.

The production of gum by the bacterium from any of these celluloses would assume, firstly, that the organism secreted a cellulose-dissolving enzyme, and secondly, that the products of zymolysis were capable of being utilised. In the first place there is no evidence to show that the organism does secrete such an enzyme. The presence in plants of a gum-ferment capable of converting cellulose into gum has been claimed by Weisner, but this has been contradicted by Reinitzer, who regards it as a simple diastase.

In the second place, many of the celluloses give dextrose or galactose only upon hydrolysis, and such would be useless as gum-producers. Levulose is rarely produced, but when yielded by certain hemicelluloses‡ the accompanying dextrose would prevent

* Tollens, Abstract in Journ. Soc. Chem. Ind. 20, 740.
† Oppenheimer, Ferments and their Actions, 189.
‡ Schulze und Castoro, Biochem. Cent. i. 785.

its utilisation. With regard to the sugars, mannose, arabinose, and xylose I cannot speak with certainty, as I did not have them in the laboratory when the experiments were made. In view of the fact, however, that levulose produced so much slime experimentally, and that it is the chief wandering sugar,* there is no reason for supposing that the celluloses contribute to the smallest extent in the nutrition of the organism, even if by some means they should become hydrolysed. Maltose, the other wandering sugar, has been shown to be active in producing gum.

The ideas regarding the cellulose origin of the gum have doubtless arisen in part from the occasional finding of the gum in pockets in the trees. Lutz† found gum-reservoirs in the bark and pericycle of acacia. These consisted of lacunæ caused by the enormous swelling and ultimate deliquescence of the cell walls. It is extremely probable that the solution of the cell walls was caused by micro-organisms other than the gum-forming bacteria. The gum formed in the otherwise healthy vessels would naturally flow into these cavities already formed by moulds, where it might possibly increase in quantity. I have found gum in cavities in the fruits of the almond and the peach, when it had undoubtedly been formed in the stem and branches. It must also be remembered that a semi-soluble gum might increase locally in some of the vessels and rupture the tissues mechanically. This is very commonly observed in Eucalyptus trees, in which the semi-solid gum kino is formed. I have never found soluble wattle-gum in pockets which did not show evidence of grub habitation, and the phenomenon must be rare if it does occur.

The depressing action of galactose and dextrose.—In writing this part of the paper it occurred to me that galactose, one of the most common hydrolytic products of many of the celluloses such as the pectins and hemicelluloses, might not only be useless as a source of gum, but might also hinder or prevent the utilisation of other sugars by the bacterium. Dextrose might also be capable of preventing the formation of gum from maltose. These points

* Brown and Morris, Journ. Chem. Soc. 1893, Trans. 674.
† Reynolds Green, Soluble Ferments, 98.

appeared worthy of experimental enquiry, and accordingly experiments were made with the following results :—

THE INFLUENCE OF GALACTOSE UPON LEVULOSE AND DEXTROSE UPON MALTOSE.

	Slime in grams from 100 c.c. of medium;* 3 days at 19°.
Levulose 1%	22
,, ,, + galactose 2%	0
,, 2%	21
,, ,, + galactose 1%	11
,, ,, + ,, 2%	1
Maltose 1%	8
,, ,, + dextrose 1%	0
,, ,, + ,, 2%	0
,, 2%	17
,, ,, + dextrose 1%	0
,, ,, + ,, 2%	0
Maltose 1% + galactose 1%	4

The results are interesting in showing that galactose behaves like dextrose in prohibiting the slime-formation from levulose. It is rather more pronounced in its action, as can be seen by comparing the experiment with that on page 231. Dextrose prohibits the formation of gum from maltose, and since it acts similarly with levulose, there is the probability that it does so with every sugar. The same may be said of galactose, which, as shown in the single test, depresses the yield from maltose. The experiment serves to confirm what I have already said regarding the improbability of gum being derived even from the most easily attacked celluloses.

The optimum temperature.—An air temperature of about 17° had given the best results when growing slime upon saccharose-potato tannin-agar media. Further trials, however, appeared advisable, so that the weights of slime at different temperatures could be given. Three experiments were made; the first suggested the second, and a third was also made.

* Sugars variable, asparagine 0·1, tannin 0·1, asparagine 0·1, agar 2, water 100.

INFLUENCE OF TEMPERATURE.

	Grams of Slime from 100 c.c. of medium* at				
	13¼°	14°	17°	22°	30°
Experiment 1	...	21	...	18	5
,, 2	...	22	23·5	16·5	...
,, 3	24	...	24	16	...

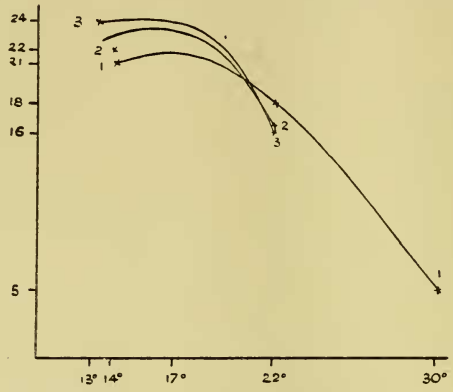


Fig. 4.—THE INFLUENCE OF TEMPERATURE.

In the first experiment the curve of the numbers showed that the optimum temperature was either about 17° or below 14°. The second indicated that the former temperature was the best and that the curve did not fall so rapidly on the lower side of the optimum as it did on the higher. The third experiment corroborated the second generally. The slime was much more rapidly formed at 22° than at 17°, the bulk being produced in 24 hours. At 17°, the slime was chiefly formed between the second and third day.

The best artificial medium.—In reviewing the numerous experiments, it appears that so long as levulose and asparagine are

* Asparagine 0·1, levulose 2·0, tannin 0·1, potassium citrate 0·1, agar 2, water 100 grms. Commercial tannin was used in No. 3.

present in a medium and a suitable temperature maintained, there is little need to have salts or other constituents. The slime may be slightly increased by the addition of traces of a citrate or a succinate, of glycerine and of tannin. The best medium for enabling *Bact. acaciæ* to form its slime in the laboratory should contain the following constituents, and the infected medium should be incubated at 17°.

Levulose..	2·0 grms.
Glycerine	1·0 ,,
Asparagine	0·1 ,,
Tannin	0·1 ,,
Potassium citrate	0·1 ,,
Agar	2·0 ,,
Water	100 c.c.

This medium is something more than a substratum for growing the slime of *Bact. acaciæ*. It is a diagnostic for the slime-forming bacteria. Some organisms, *e.g.*, *Bact. acaciæ*, form a voluminous slime upon it readily; others, *e.g.*, *Bact. metarabinum* in its highest state of development in artificial culture, produce a tough leathery growth; while others, *e.g.*, *Bac. levaniformans*, refuse to grow. Following is a list of the gum bacteria with which I have worked :—

Slime produced.	No slime produced.
Bact. acaciæ.	*Bact. levaniformans.*
Bact. metarabinum (dry, tough).	*Bact. eucalypti.*
Bact. sacchari.	*Bact. vascularum.*
Bact. pararabinum.	*Bact. persicæ.*

Gum Bacteria under investigation and not yet described.

Sunflower ii.	Sunflower iii.
Lime ii.	Lime i.
Quince ii.	Variable galactan bacterium (trace).
Macrozamia *e.*	Macrozamia *b* (trace).
Bact. pseudarabinum, n.sp.	Linseed xi. (trace).

Growth upon saccharose-potato-agar.—It will be remembered that the slime of the arabin bacteria *Bact. acaciæ* and *Bact. metarabinum* was first obtained in quantity upon a medium made with saccharose, tannin and potato-extract. At a later date, when the extract had been obtained from a second lot of potatoes, the bacteria refused to produce the slime. That this was probably not due to a deterioration of the gum-forming faculty of the bacteria was inferred from the formation of a galactan slime of *Bact. sacchari* upon the old potato medium after this organism had been subcultivated in the laboratory for over a year. The potato-extract was apparently at fault, and experiments which were made showed that when a smaller quantity of the extract was used in the agar medium the bacteria again produced their slimes. At a still later date a third lot of potatoes were bought and the extract prepared. Upon media prepared with this extract no slime could be obtained, and it made no difference how much the raw juice was diluted. I was of the opinion that the behaviour of the second lot of potatoes might have been due to the fact that they were new, the first quantity having been obtained in the winter and the second in the spring. But as the third purchase made was in the following winter, and to all appearance was similar to the first, the quality as determined by winter and spring tubers could not explain the difference in the slime-forming effect. In Australia there is no great difference between potatoes, such as there is in the British Isles, for the climate forbids their storage, and they reach the Sydney market during the year from localities at different elevations and in different latitudes.

A number of experiments were made with the third potato-extract medium, such as varying the tannin and the acidity, but to no purpose. However, about this time the influence of dextrose in depressing the slime-forming faculty was discovered, and it appeared to be extremely probable that an excess of dextrose in the extract or juice was the real reason for the non-production of gum by the bacteria.

An experiment was made in which levulose was added in varying amounts to the potato medium, and the following results were obtained :—

THE ADDITION OF LEVULOSE TO POTATO-EXTRACT.

	Slime in grams from 100 c.c. of medium.*
No levulose	None
Levulose 1%	8
,, 2%	15
,, 3%	21
,, 5·3%	23

The production of 21 or 23 grms. of slime shows that the medium contained a sufficiency of asparagine and salts, and the yield of 8 grms. with 1% levulose and 15 grms. with 2% shows a depression such as might be caused by the presence in the medium as prepared of about 1% of dextrose (see p. 231). The depressing constituent of the potato-juice might have been held accountable for the non-production of slime from saccharose had it not been discovered towards the close of this research that the bacteria could again utilise the sugar. The power of forming slime from saccharose was thus a faculty which the organism could gain and lose, and to maintain this power it had to be rapidly subcultivated upon saccharose media, that is, the transfers had to be made every second or third day. Upon referring to my notes I found that, subsequent to the work connected with the first two papers of this series, the bacteria had been subcultivated upon ordinary nutrient agar which contains no saccharose. From the beginning of this research I had grown them upon saccharose-potato-agar, and on this medium the power of using saccharose had been regained.

Slime from molasses.—Bearing in mind the possibility of arabin being in the future manufactured industrially from certain waste

* Potato-juice 10, tannin 0·1, agar 2, water 90 grms.

products such as molasses or the liquor from potato starch, I tried the effect of adding molasses to agar. The sample of molasses contained the following constituents:—cane sugar 38%, fruit sugar 9%, other organic matters 16%, soluble ash 10%, and water 27%. The most favourable yield of slime was about half of what would have been obtained had saccharose and asparagine been employed. Either the dextrose or the excessive saline matter prevented a greater production, but it is also possible that the nitrogen was deficient. Molasses contains from 0·013 to 0·027% of nitrogen, which, being calculated to asparagine, would mean 0·07 to 0·144%; the medium with 5% of molasses would thus contain the equivalent of from 0·0035 to 0·0072% of asparagine, which is undoubtedly too small. Experiments were made with urea on account of its cheapness industrially, and these showed that nothing was to be gained by adding it to the molasses. The addition of potato-juice in varying quantities was also inoperative. The presumable deficiency of nitrogen could not therefore play a part in the lessened yield, and the other constituents of the molasses must be considered as being the active agents. With small quantities of molasses (2% and under) an ordinary infection as by smearing the plates in the manner already described was sufficient, but with larger quantities a mass-infection was absolutely necessary to obtain a yield of slime. This was done by smearing a small loopful of culture (made upon saccharose-potato-agar at 30°) over an area of about a quarter of an inch diameter in the middle of the plate. In 24 hours the slime which had formed was spread over a wider area of about an inch diameter. In another 24 hours the area was increased to 2 inches, and in another day the slime was sufficient to enable the whole plate to be smeared. The slimes were scraped from the plates on the eighth day and weighed. In the following table four experiments are tabulated:—

Molasses as a Source of Gum.

Percentage of tannin in medium.	Slime in grams from 100 c.c. of medium.*			
	i.	ii.	iii.	iv. (17° C.)
				...
0·2	0·0	...	...	...
0·25	...	0.1	...	...
0·4	0·2	...	..	...
0·5	...	0·5	...	...
0·6	1·3	...	...	...
0·8	3·0	...	...	...
1·0	3·7	5·0	...	...
1·2	4·6	...	...	...
1·4	6·9	...	...	...
1·5	...	8·5	5	...
1·6	9·0	...	..	...
2·0	10·1	11	8	...
2·5	...	12	10	...
3·0	...	11	10	15
3·5	...	13	12	15
4·0	...	12	11	16
4·5	...	16	13	18
5·0	...	11	12	16
5·5	...	...	...	16
6·0	...	...	13	15
6·5	...	...	12	16
7·0	...	...	10	15
7·5	...	..	...	16
10·0	...	..	11	...

The experiments show that slime can be obtained from molasses, although there are some constituents (probably dextrose and salts) that are inimical to the full development of the bacteria. These check the growth unless a mass-infection is given and give from one-half to two-thirds the maximum yield of slime. It also appears to be immaterial how much molasses is employed in the medium within certain limits, although 4·5% seems to be an optimum amount.

Precaution in growing slime upon artificial media.—One observation was made in connection with the production of slime in tubes of media. When a certain medium had been recently prepared, slime was readily formed by the bacteria, but after the

* Molasses varying quantity, tannin 0·1, agar 2·0, water to 100.

lapse of about a month not only could no slime be formed, but the cells refused to grow. A few experiments showed that the reason for this was that the surface of the agar had become dry during the interval that passed between the sloping of the tube and its utilisation. The medium must therefore be recently prepared if a production of slime is desired.

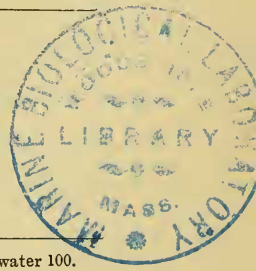
The possibility of curing the gum-flux of trees.—In considering the possibility of being able to prevent or check the formation of gum by the bacteria in the tissues of plants, it is evident that we have to deal with organisms that flourish in poor substrata. A small quantity of a suitable sugar ($\frac{1}{4}\%$) and of a nitrogenous substance ($\frac{1}{25}\%$) are enough for the bacteria to produce half the maximum amount of gum. Rich substrata rather tend to prevent gum-formation; this has been seen in the effect of sugars and salts. It has been noted that gum is found upon trees in unhealthy surroundings. Such conditions would reduce the composition of the sap below the normal, and while the growth of the tree was hindered the growth of the bacteria would be accelerated. The influence of temperature is very pronounced, and a reference to the quantity of slime produced at different temperatures is enough to show how the formation of gum is increased by a cold season or by the presence of the tree in a damp, cold hollow. In the latter cases the drainage will have an influence in lessening the vitality of the tree. In all questions of invasion of plant tissues by bacteria it is a rule that a healthy plant will overcome the attacks of the organisms, and it probably does this by itself utilising the products of its own metabolism, thus starving the bacteria, and also by maintaining the acidity of its juices. The success of the plant is also probably influenced by the small numbers of invading bacteria, for a large infection would enable the organisms to get a hold which would lead to the defeat of the plant. A mass of bacteria will secrete more byproducts than a few cells, and these being localised in the host plant may injuriously affect the cells in the locality, and thus enable the bacteria to establish themselves.

In checking the formation of gum on valuable plants, such as fruit trees, the healthiness of the tree should first be considered and means taken to ensure the efficient drainage of the soil and the absence of decaying vegetable matter such as buried stumps. The proper manuring of the tree will next suggest itself so that the plant might be stimulated to a healthier condition. The excision of the part or parts from which the gum is oozing will probably be of little avail, because it is probable that the bacteria are producing the gum in the tissues between the wound and the growing point of the stem or branch. If the gum should exude from a branch only, then the pruning of that branch would probably be beneficial.

Any chemical remedy for the prevention of gum-formation must consist in the application of a salt injurious to the bacteria. The effect of a nitrogenous salt would be indirect. Other salts might act directly, and with regard to these something might be learned from laboratory experiments. We have seen that a trace of a succinate or citrate is beneficial, and that some salts were indifferent, while others were injurious. But in the former tests an optimum medium was not employed, and it, therefore, appeared to be wise to repeat the experiment by adding salts to the optimum medium and noting the depression which they exerted upon the yield of slime. Accordingly tubes of the optimum medium were prepared and salts added to the extent of 0·5%.

The effect of Salts upon the Optimum Medium at 17° C.

Salt added.	Grms. of slime from 100 c.c. of medium.*
No salt	26
Potassium chloride	19
Sodium ,, ...	15
Calcium ,, ...	16
Magnesium sulphate	16
Sodium phosphate	17
Magnesium sulphate ... ... } Calcium chloride ... ... }	17
Potassium nitrate	12
,, sulphate	20



* Levulose 2, asparagine 0·1, potassium citrate 0·1, tannin 0·1, agar 2·0 water 100.

From the results it was clear that common salt or potassium nitrate were the most promising for further experiment. Varying amounts of these were therefore added in a subsequent trial. Owing to pressure of other work this was done six weeks afterwards, and during the interval the bacteria had been resting and had partly lost their slime-producing faculty, as will be seen from a comparison of the amounts formed on the control media. During the interval the laboratory temperature had risen to about 22° in Experiment i. and to about 20° in Experiment ii.

The depressing influence of Sodium Chloride and Potassium Nitrate.

	Slime in grms. from 100 c.c. of media.*	
	i.	ii.
Control—no salt	16	20
Sodium chloride 0·2 %	14	17
,, ,, 0·4 ,, ...	12	12
,, ,, 0·6 ,, ...	—	8
,, ,, 0.8 ,, ...	5	4
,, ,, 1·2 ,, ...	3	1
Potassium nitrate 0·2 ,, ...	9	8
,, ,, 0·4 ,, ...	—	7
,, ,, 0·8 ,, ...	10	8
,, ,, 1·2 ,, ...	11	9

From the experiments it is unlikely that anything would be gained by manuring the trees with salts such as common salt, for the quantity which would affect the bacterium would also injure the plant. But possibly something might be gained by the use of small quantities of potassium nitrate, which would act as a plant stimulant and as a bacterium depressant. Field experiments would alone determine this question.

Infection experiment with peach trees.—It is customary to identify the relation of an organism with a particular disease, whether of plants or of animals, by introducing a pure culture of

* Levulose 2·0, tannin 0·1, asparagine 0·1, potassium citrate 0·1, agar 2·0, water 100.

the organism in question into the tissues of the host. By the reproduction of the symptoms of disease the etiological relationship is established. This method serves very well in the case of animals in which toxic symptoms can be readily observed, and especially as the toxic products of the bacteria cannot as yet be identified chemically. But with plants it is different. The toxic effects are not as a rule decided enough to convince the sceptic, and not unfrequently control plants develop disease as readily as the inoculated.

In my work with plant diseases I have always endeavoured to produce the typical bacterial product in the laboratory and to compare it chemically with the substance formed in the host plant. This is, of course, not always capable of being done, but in the present instance the production of arabin in the laboratory by *Bact. acaciæ* is a much more decided proof that the bacterium produces arabin in the plant than would be the production in the plant after an infection with the bacterium, for the simple reason that we could not be absolutely certain that the tree would not have developed gum independently of the infection, possibly as the result of a previous or a subsequent accidental infection with the same or another organism.

Still I made an infection experiment, but it was done more with the idea of testing whether the bacterium of the wattle could produce gum in the peach, the only susceptible tree which chanced to be in my garden. The trees were about 5 feet high, and were infected at places about $2\frac{1}{2}$ feet from the ground. Three trees were selected. One of them forked at 2 feet, and one fork was infected with *Bact. acaciæ*, the other with *Bact. metarabinum*. A second tree was inoculated with *Bact. acaciæ*, and a third with *Bact. metarabinum*.

Upon returning after the summer vacation the trees were carefully examined and gum was found in two cases. One was the tree infected with both bacteria, the other that which had been inoculated with *Bact. acaciæ*. The third, infected with *Bact. metarabinum* only, had no gum. The forked tree had been inoculated with the two bacteria to see if different gums would be

produced in the different branches, but as the gum upon both trees had exuded at a place about two inches from the ground, the experiment, so far as this question was concerned, was a failure. The gum was protected with thin rubber tissue, but this shortly perished, and the gum was washed away* by the rain.

Several other trees near the experimental ones showed no signs of gum-flux, so that it may be accepted, as shown by this field experiment, that *Bact. acaciæ* can produce gum-flux of the *Rosaceæ* as well as of the *Acaciæ*. It is interesting to note that the gum in this case descended the stem, which would point to the majority of the bacteria travelling in the descending current, probably in the cellular tissue of the bark, the place in which levulose and maltose, the sugars of translocation, would be found. There is, of course, the possibility that the gum exuded low down on the stem in both cases owing to wounds being there, and there only, but as the branches had at an earlier time been freely pruned below and above the site of infection this objection is scarcely tenable.

One year later the fork of the tree which had been infected with *Bact. metarabinum* showed signs of gum in several places, and an oval piece of gum measuring 2 × 1 cm. and 2 mm. thick in the centre was removed. A portion was tested and found to be metarabinum.

The third tree that had been infected with *Bact. metarabinum* only showed a considerable gum-flux at a place where a wire chafed the stem. The gum was of the metarabin variety. It is curious that the branch in one case and the tree in the other, both of which had been infected with the metarabin bacterium, produced this gum a year after inoculation, and that control trees showed no sign of gum.

In January, 1904, one of the check trees of the previous year was infected with a culture of *Bact. acaciæ* which had been growing in the laboratory for over a year, and with which most of the experiments concerning the nutrition of the organism had been

* Metarabin gums swell enormously with water and fall off.

made. Two months afterwards the tree was examined, and small pellets of yellowish gum were found at the infected places. In this case the gum had not migrated down the stem. The granules of gum simply swelled with water and did not dissolve, thus showing it to be metarabin. It is possible, and I think it quite probable, that as the gum issued from the wounds it consisted of a mixture of arabin and metarabin, and that occasional rains had dissolved the former and washed it down the trunk of the tree. Control trees show no appearance of gum, and, therefore, we must conclude that the exudate was caused by the infected bacteria.

Bact. metarabinum, a variety of *Bact. acaciæ*.—When we remember that *Bact. acaciæ* and *Bact. metarabinum* virtually differ in the production of arabin and metarabin respectively,* it is significant that *Bact. acaciæ* should, when infected into the peach, produce a gum-flux of insoluble metarabin instead of the soluble arabin. The gums of the Rosaceæ are practically always metarabin (or cerasin, as it is frequently called), and this experiment shows that the host tree can alter the gum-forming faculty. This it must do either by modifying the gum after its formation by *Bact. acaciæ* or by modifying the bacterium, so that it becomes a metarabin producer, *i.e.*, *Bact. metarabinum*. To gain some information concerning this, I examined the tissues of a branch of the infected tree. The branch sprang from a place between the infected areas of the stem. In the plates of glucose gelatine that were prepared there developed colonies of *Bact. acaciæ*, and upon one of the plates I obtained an impure colony of *Bact. metarabinum* growing upon an imbedded fragment of bark. I have already† discussed the difficulty experienced in isolating *Bact.*

* With the exception of the gum, the bacteria form the same products during the fermentation of saccharose, and they are morphologically very similar. If the gums had an equal solubility all the cultural characters would probably be identical. In short they appear to be races of one organism producing different kinds of gum. But the gums so modify the characters of the cultures that the races appear to be species. Believing this to be the case, I subcultivated both bacteria at 17° and at 30° for four months to see if the characters would approximate, but no change occurred.

† These Proceedings xxvii. (1903), 126.

metarabinum on account of its property of remaining in clumps, while *Bact. acaciæ* diffuses throughout the culture medium. The finding of even one colony of *Bact. metarabinum* was therefore quite sufficient, for there might have been, and probably were, more original bacteria in the one colony of *Bact. metarabinum* than in all the colonies of *Bact. acaciæ*. The impurity in the original colony consisted of *Bact. acaciæ* and a form intermediate between it and *Bact. metarabinum*, that is a transition form between the two. Upon glucose gelatine the colonies of the transition form grew first like *Bact. acaciæ* and then became puckered like *Bact. metarabinum*.

It has by this experiment been shown that the host plant can alter the physiological function of *Bact. acaciæ*, and that so profoundly that the acquired character is practically permanent. In the case of the bacterium from *Acacia penninervis*, the formation of metarabin has been maintained for two years, during which the organism was subcultivated in the laboratory. Since both bacteria were found in the tissues of the tree, it is probable that the exudate really did consist of a mixture of the gums, and that taken from the wounds was really the metarabin residue. The fact that a tree can alter the gum-forming function explains how the gum from different species of trees are so constant in their characters.

The growth of Bact. metarabinum.—Experiments were made with *Bact. metarabinum* to determine the nutrients that favoured the production of slime when the research with *Bact. acaciæ* was nearing completion. Like the preliminary experiments with the latter, the first trials were abortive owing to the lengthened subculture of the organism upon nutrient meat-agar. But after a few rapid transfers upon saccharose-potato-agar* the bacteria rapidly regained the power of forming slime. This was, however, so insoluble that it could not be removed from the agar even when made with 0·3% of tannin. As the gum is not readily

* The saccharose-potato-agar which I now employ consists of potato-extract 250 c.c., 50% solution of saccharose (autoclaved to free it from *Bac. levaniformans*) 40 c.c., agar 20 grams, and water to 1000 c.c.

formed in fluid cultures, the organism did not lend itself to determinative experiments, such as those that had been made with *Bact. acaciæ*. Still so far as they went, the appearances of the agar cultures seemed to show that what favoured the one organism also favoured the other.

The rapid subcultivation upon saccharose-potato-agar produced a growth which indicated that the gum was more insoluble than at the time when the cultural characters of *Bact. metarabinum* were described. At what may be called the height of its development, that is when the gum is most insoluble, the growth on the different media are much drier and more wrinkled than have been described. The colony on glucose gelatine as illustrated is typical of the organism after it has been cultivated for a short time in the laboratory, say from the 5th transfer onwards. Up to the 5th subcultivation the colony has not the regular wrinkled formation, but appears as an irregular moruloid colony. The coarsely granular structure of the glucose-gelatine colonies of *Bact. acaciæ* is apparent, even in the colonies upon the first, *i.e.*, the original plate. A typical colony of *Bact. pararabinum* has been reproduced in order to complete the set of the arabin bacteria. It is by the appearance of the colonies on nutrient glucose gelatine that the bacteria can be most easily diagnosed.

The conclusions that may be drawn from the research are as follows :—

1. *Bacterium acaciæ* can produce gum readily in the presence of suitable nutrients.
2. Levulose and saccharose are the best sources of carbon; maltose, mannite and glycerine come next, whilst dextrose, galactose, lactose and raffinose are of no use.
3. The organism acquires and readiily loses the power of utilising saccharose.
4. Dextrose or galactose prevents the gum being formed from levulose or maltose.
5. The bacterium temporarily loses the power of forming gum when subcultivated upon sugar-free media.
6. Molasses can be employed for the production of gum.

7. Amides are the best nitrogenous nutrients; peptone is of little use. A trace of asparagine is sufficient.
8. Salts may accelerate, depress, or prevent slime-formation. Traces of alkaline citrate or succinate were most favourable.
9. Sumach tannin assists the formation of slime upon artificial agar media. Oak tannin hinders the formation, but the retarding effect may be neutralised by the addition of glycerine.
10. The bacterium might be used to distinguish certain tannins.
11. Tannin probably acts physically by making the agar medium more contractile, so that the organisms are slowly supplied with nutrients in solution.
12. The optimum temperature is 17° C.
13. The most suitable medium, as deduced from the experiments, serves as a diagnostic for other slime bacteria.
14. Gum acacia has not a cellulose origin.
15. In the host plant it is formed from the wandering sugars, levulose and maltose.
16. Manuring with saline matter does not promise to be a remedy for the prevention of gum-flux in fruit trees.
17. Peach trees that were inoculated with *Bact. acaciæ* (from *Acacia binervata*) developed gum-flux.
18. The exudate was a metarabin gum.
19. The host plant can convert *Bact. acaciæ* into *Bact. metarabinum*, proving what had been suspected, that the latter is a variety of the former producing an insoluble gum.
20. This explains the uniformity of the gums from certain species of trees.

EXPLANATION OF PLATES XI.-XII.

Colonies of the arabin group bacteria upon nutrient glucose-gelatine.

Plate xi.

Fig. 1.—*Bact. acaciæ* (× 30).

Fig. 2.—The same organism twelve months afterwards (× 30).

Plate xii.

Fig. 3.—*Bact. metarabinum* (× 38).

Fig. 4.—*Bact. pararabinum* (× 22).